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Polygalacturonases of a latent and wound postharvest fungal pathogen of muskmelon fruit

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Abstract

Partially purified endo- and exo-polygalacturonases (PG) from two fungal pathogens (*Phomopsis cucurbitae* and *Rhizopus stolonifer*) were compared in relation to their ability to macerate netted muskmelon tissue at different stages of fruit development. PG extracts from *P. cucurbitae*, a latent infection pathogen, produced little maceration until fruit were 50 days post-anthesis (10 days postharvest). In contrast, PGs from *R. stolonifer*, a wound pathogen, produced high levels of maceration at all stages of fruit development from 20 to 50 days post-anthesis. Both pathogens demonstrated highest levels of total PG activity in mesocarp and lowest levels in exocarp (peel) tissues. Isoelectrofocusing–polyacrylamide gel electrophoresis indicated two prominent PG isozymes in *R. stolonifer* and nine isozymes in *P. cucurbitae*. Cell wall carbohydrate analysis showed an approximately 6-fold decrease in galactosyl residue content between 10 and 50 days post-anthesis in uninfected fruit. Infected fruit showed approximately 7- and 8-fold decreases in galacturonic acid content when infected with *P. cucurbitae* and *R. stolonifer*, respectively. Significant decreases in cell wall rhamnosyl and arabinosyl residues occurred during infection of fruit with both pathogens. These results support a role for cell wall pectin degradation during the decay process of muskmelon by these pathogens. The ability to macerate fruit tissue, as related to the latent infection phenomenon, may be due to substrate specificity or inhibitors present in muskmelon fruit tissue.

Keywords: Cantaloupe; Cell wall; *Cucumis melo* var. *cantaloupensis*; Fungal decay; Inhibitor; Isozymes; Pectinases; *Phomopsis cucurbitae*; Postharvest; *Rhizopus stolonifer*

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1. Introduction

Muskmelons (*Cucumis melo* var. *cantaloupensis* Naud.) are an important horticultural crop in the United States. The muskmelons produced are primarily for domestic consumption, although significant quantities are exported to Canada. The main factor limiting export is the short shelf-life and development of associated postharvest decays (Bruton, 1995). However, there have also been successful shipments of netted muskmelon to Europe using a modification of the shrink-wrap technique developed by Lester and Bruton (1986).

Hard-ripe muskmelon have a normal postharvest shelf-life of about 14 days under proper storage conditions (Lester et al., 1988; Lester and Stein, 1993). However, fruit firmness declines by 16% after 10 days storage and 37% after 20 days storage (Lester and Dunlap, 1985; Lester, 1988). As muskmelon fruit soften, postharvest decays increase significantly (Bruton, 1995). A number of species of *Fusarium* are involved in one of the most important postharvest diseases of netted muskmelon (Bruton, 1995). Incipient infections that pass undetected through the grading and packing process are of primary concern in postharvest decay. *Phomopsis cucurbitae* Mckeen, the anamorph of *Diaporthe melonis* Beraha and O'Brien, is strictly a latent infection fungus and is second only to *Fusarium* spp. in importance on muskmelon produced in the southern US (Bruton, 1995, 1996a). The fungus *Rhizopus stolonifer* (Ehrenb. ex Fr.) Vuillemin can be extremely destructive if the melons have been injured during harvesting and packing (Bruton, 1996b). Ceponis et al. (1986), summarizing United States Department of Agriculture, Agricultural Marketing Service data on nearly 9000 muskmelon shipments arriving in New York City between 1972 and 1984, reported that *R. stolonifer* ranked fourth in frequency among the identified decays.

Pectolytic enzymes of fruit decay pathogens are thought to play a major role in tissue maceration (Bateman and Basham, 1976; Huber, 1983; Swinburne, 1983). Numerous fungal plant pathogens produce pectic enzymes during invasion of fruit tissue (Bateman, 1968; Conway, 1982; Cooper, 1983; Tobias et al., 1995). Comparing the macerating ability of polygalacturonases (PG, EC 3.2.1.15) from a latent infection and wound pathogen could help explain the mechanisms of latent infection. Thus, the objectives of this study were to compare *P. cucurbitae* (latent infection) with *R. stolonifer* (wound pathogen) in terms of their ability to decay fruit, the ability of the enzyme extracts of the fungi to macerate fruit tissues, and the PG isozyme composition of the fungi. In addition, we studied muskmelon cell wall composition of the exocarp with respect to fruit age and tissue maceration, and breakdown products during decay of fruit tissue by the two fungi.

2. Materials and methods

2.1. Fruit decay

Thirty healthy muskmelons were inoculated with *P. cucurbitae* or *R. stolonifer* after surface disinfecting the fruit with 80% alcohol, and removing tissue plugs with a cork borer (approximately 1 cm diameter and 1 cm deep). An inoculum plug, from 4-day-old potato dextrose agar cultures was placed upside down in the fruit and sealed with molten paraffin. Each melon was inoculated at two sites on opposite sides and stored at 25°C for 4 days. Each melon was cut perpendicular to the point of inoculation and the profile of the lesion was traced on a transparency sheet. The area of fungal decay was calculated using an area meter (Li-Cor, Lincoln, NE, USA) and analyzed as a completely randomized one-way experiment.

2.1.1. Extraction and partial purification of PG enzymes

Pectolytic enzymes were extracted from *P. cucurbitae* and *R. stolonifer* to determine their ability to macerate muskmelon fruit tissue. Tissue from naturally infected muskmelon fruit was removed and frozen in 100-g samples at −80°C. All subsequent extraction operations were carried out at 4°C. Tissue was thawed and put into a homogenizing buffer containing 20 mM MES, 1 M NaCl, and 5% polyvinyl–polypyrrolidone, pH

6.0. The entire mixture was homogenized for 1 min using a polytron and strained through two layers of cheesecloth. The filtrate was centrifuged at $40000 \times g$ for 25 min. The supernatant was concentrated using a Minitan ultrafiltration system (Millipore, Bedford, MA, USA) to approximately 50 ml and subsequently filtered through 0.2 μm Gelman filter paper (Gelman Sciences, Ann Arbor, MI, USA). The supernatant was loaded onto a cation exchange column (CM-Sephadex C-25, Pharmacia, Piscataway, NJ, USA) using a peristaltic pump at a flow rate of 0.5 ml/min. The enzyme was eluted with a linear gradient from 0 to 0.7 M NaCl in 20 mM MES (pH 6.0) and 5-ml fractions were collected. Following this elution, fractions exhibiting PG activity were combined, dialyzed, and concentrated. The partially purified PGs of *P. cucurbitae* and *R. stolonifer* showed specific PG activity of 83.5 and 32.1, respectively.

2.2. PG activity and protein assays

PG activity was assayed by measuring an increase in reducing sugars with 2-cyanoacetamide described by Gross (1982). In the present study, no experiments were performed to determine if the activities measured represented exo- or endo-PG action, although this may be important for future studies. Reaction mixtures contained 100 μl of 1% polygalacturonic acid (Sigma Chemical Co., St. Louis, MO, USA), which was washed with 80% ethanol before use to remove oligosaccharides, in 20 mM MES buffer (pH 6.0), 50 μl of distilled water and 50 μl of sample. Following incubation for 20 min at 37°C, the reactions were terminated by adding 1 ml of cold 100 mM borate buffer (pH 9.0). Then, 200 μl of 2% 2-cyanoacetamide were added, the sample mixed, and immersed in a boiling water bath for 10 min. After cooling on ice, the absorbance at 276 nm was determined. Galacturonic acid was used to establish a standard curve. One unit of PG activity was defined as the amount of enzyme required to release 1 μmol of reducing groups per minute at 37°C. In all cases, product formation was linear with time and proportional to the amount of enzyme in the reaction mixture. The protein content of the enzyme extract was determined using bicinchoninic acid (Smith et al., 1985) with bovine serum albumin as a standard.

2.3. Isoelectrofocusing

Isoelectrofocusing–polyacrylamide gel electrophoresis (IEF–PAGE) (T, 7%; C, 3%; 0.5 mm thickness) containing 2% (v/v) ampholine (pH 3.5–10) was cast using an IEF kit from Pharmacia (Piscataway, NJ, USA). Concentrated enzyme samples were applied to each application piece (20 μl/rep) and then removed from the gel after 20 min. The gel was run on a Multiphor II apparatus (Pharmacia) at a constant power of 20 W and a maximum of 2000 V for 2 h including 20 min of pre-isoelectric focusing. After the run, PG isozymes were detected by overlaying the running gel with a pectate–agarose gel containing 1% agarose and 0.1% polygalacturonic acid with 50 mM sodium acetate, pH 5.0. Incubation and staining procedures were conducted according to Brown et al. (1992).

2.4. Fruit tissue maceration

Approximately 20 muskmelons (cv. 'Magnum 45') were harvested at 20, 30, 40 (horticultural maturity), and 50 days (harvested mature and stored at 4°C for 10 days) post-anthesis. All fruit were washed with water, and then 20–50 plugs were taken from each melon at random using a cork borer. Each piece was divided into four sections; the exocarp being the outer 1–2 mm of the fruit, and the remainder divided into three equal sections and labeled outer, middle, and inner mesocarp. All pieces were blotted dry with a damp sponge, weighed, and grouped into samples of approximately 2 g.

A minimum of five replications were used for each tissue type and for each fungal enzyme test. Partially purified PG from *P. cucurbitae* and *R. stolonifer* were used as well as a standard (pectinase, Sigma Chemical Co.) and a control blank (20 mM MES pH 6.0). All enzymes were adjusted to an activity of 0.3 unit/ml; then 5 ml of each substrate were added to each tissue sample and incubated, without shaking, at 25°C for 4 h. Tis-

sue was removed from each treatment and weighed to the nearest milligram. Percentage weight loss was calculated. The effects of fruit tissue type, fungal enzyme, and maturity were analyzed. Data were analyzed as a completely randomized three-factor experiment. Since the three-way interaction of tissue type, enzyme, and fruit age were significant, further evaluation of tissue response to enzyme with respect to fruit age was subjected to covariance analysis. In this way, it was possible to test for differences in tissue maceration of different age fruit and fungal enzyme by comparing regression slopes and intercepts of percentage tissue loss regressed over fruit part (scaled as exocarp = 0, outer mesocarp = 1, middle mesocarp = 2, and inner mesocarp = 3).

2.5. Cell wall carbohydrate composition

Approximately 30–50 muskmelon fruit were harvested at 10, 20, 30, 40 and 50 days post-anthesis. Fruit exocarp was cut to a depth of 1–2 mm and the remainder of the fruit discarded. Three samples of 1 kg each were ground in 80% EtOH (1:2 w/v) for 1 min using a homogenizer (Kinematica, Littau, Switzerland) and stored at −20°C. The homogenate was filtered through Miracloth (Calbiochem-Behring, La Jolla, CA, USA) and the residue washed with distilled water. Walls were re-suspended in 20 mM HEPES buffer (pH 7.6) and homogenized for 1 min. Cell walls were filtered again and washed with 2 L of buffer three times. Cell walls were then suspended in 1.5 L of HEPES buffer containing 1 unit/ml amylase (Sigma Chemical Co.) and three drops of toluene. The suspension was incubated for 18 h in a shaking water bath (30 rpm, 30°C) to remove starch. However, starch is not known to be a component of muskmelon fruit. Cell walls were subsequently filtered and rinsed with 1 L of buffer and re-suspended in phenol/acetic acid/distilled water (2:1:1; w/v/v) to inactivate cell wall-associated enzymes. The slurry was stirred for 30 min, filtered through a sintered glass filter and re-suspended in 2 L chloroform/methanol (1:1; v/v) and stirred for 10 min. The residue was filtered, washed with 1 L chloroform/methanol, re-suspended in 1 L acetone, stirred slowly for 5 min, filtered and washed again with acetone. Cell wall material was dried over P_2O_5 in vacuo at 25–37°C to a constant dry weight and then used for carbohydrate composition analysis, using the method described by Gross and Sams (1984). Regression was used to examine levels of carbohydrates during fruit development.

2.6. Cell wall carbohydrate composition of decayed tissue

Muskmelon fruit were harvested from field plots at full-slip maturity. Twenty fruit were inoculated, as previously described, with *P. cucurbitae* or *R. stolonifer* and incubated at 23–25°C. After 7 days, the decayed tissue was recovered and cell wall carbohydrate composition was determined as described above. Carbohydrate components from non-inoculated control, *P. curcurbitae*, or *R. stolonifer* decayed tissue were compared using a completely randomized one-way analysis of variance.

3. Results

3.1. Fruit decay

Decay symptoms, resulting from inoculation by the respective fungi, were similar to those caused by natural infections. Area of fruit decay in inoculated mature muskmelon fruit was significantly ($P < 0.01$) different with respect to the fungus. *R. stolonifer* produced over 55.8 cm^2 of decayed tissue in 4 days as compared to 11.8 cm^2 for *P. cucurbitae*.

3.2. PG activity and isozymes

Both *R. stolonifer* and *P. cucurbitae* produced PG in decayed muskmelons. Four days after inoculation, PG extracted from fruit macerated by *R. stolonifer* and *P. cucurbitae* had specific activities of 0.05 and 2.1 units/mg protein, respectively. Two PG isozymes (p*I* 6.2 and 7.9) were detected in tissue decayed by *R. stolonifer* (Fig. 1). Nine PG isozymes of *P. cucurbitae* with a wide pI (3.8–8.3) range were visualized on IEF–PAGE

followed by an agarose–pectate overlay. In addition, two major PG isozymes (p*I* 6.4 and 7.7) were detected in a commercial pectinase obtained from Sigma Chemical Company. No PG activity or isozymes were detected in an equal quantity of healthy, non-inoculated muskmelon fruit tissue.

3.3. Fruit tissue maceration

Muskmelon fruit tissue, sampled at 20, 30, 40 and 50 days post-anthesis, was subjected to partially purified PG from either the commercial pectinase standard, *R. stolonifer*, or *P. cucurbitae*. Maceration of the inner mesocarp, middle mesocarp, outer mesocarp, and exocarp exhibited a similar trend for the commercial pectinase standard and *R. stolonifer* PG in 20–50 day post-anthesis fruit (Fig. 2). The slopes of the regression lines (Table 1) for the various ages of fruit and fungal enzyme were similar for the standard and *R. stolonifer* PG. The maceration pattern of the *P. cucurbitae* PG was distinctly different from that of the commercial standard and *R. stolonifer* PG on fruit tissue up to 50 days. *P. cucurbitae* PG had significantly less ($P \leqslant 0.05$) activity on each tissue type from 20-, 30- and 40-day post-anthesis fruit, compared to the commercial standard and *R. stolonifer* PG. However, with tissue from 50-day post-anthesis melons, the maceration pattern was similar for *P. cucurbitae*, *R. stolonifer*, and the commercial standard. In the control, maceration regression lines had almost no slope, except for the inner-mesocarp of 50-day post-anthesis fruit where approximately 13% maceration occurred.

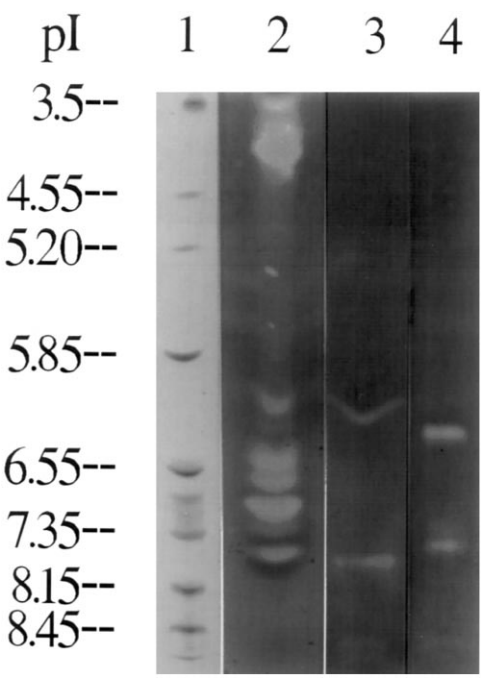

Fig. 1. Isoelectrofocusing–polyacrylamide gel electrophoresis of polygalacturonase (PG) isozymes produced by *Phomopsis cucurbitae* (lane 2) and *Rhizopus stolonifer* (lane 3) decayed muskmelon fruit. Lane 4 indicates PG isozymes of the commercial standard. Lane 1 represents pI markers.

3.4. Cell wall glycosyl composition

Levels of rhamnosyl, xylosyl, mannosyl, and non-cellulosic glucosyl residues, as well as cellulose in muskmelon exocarp cell walls, did not change significantly throughout fruit development (Table 2). However, levels of arabinosyl residues increased as fruit expanded and then decreased at 40 days. Galactosyl residues were the only wall constituent that decreased significantly throughout fruit development, decreasing approximately 6-fold from 10 to 50 days post-anthesis. Total cell wall carbohydrate content increased initially in 10–20-day-old fruit, and then decreased 15% from 20 to 50 days post-anthesis.

3.5. Cell wall glycosyl composition of decayed tissue

Compositional analysis of muskmelon fruit tissue decayed in situ by the respective fungi revealed substantial qualitative and quantitative differences in cell wall degradation products (Table 3). Fungal decay of muskmelon mesocarp by *P. cucurbitae* and *R. stolonifer* resulted in approximately 6- and 7-fold decreases in galacturonic acid content, respectively, compared to non-decayed tissue. Decay by each pathogen also produced a decrease in rhamnosyl and arabinosyl, and to a lesser extent galactosyl, residue content. An increase in relative mannosyl and non-cellulosic glucosyl content was observed in fungal-decayed tissue compared to the non-decayed control tissue.

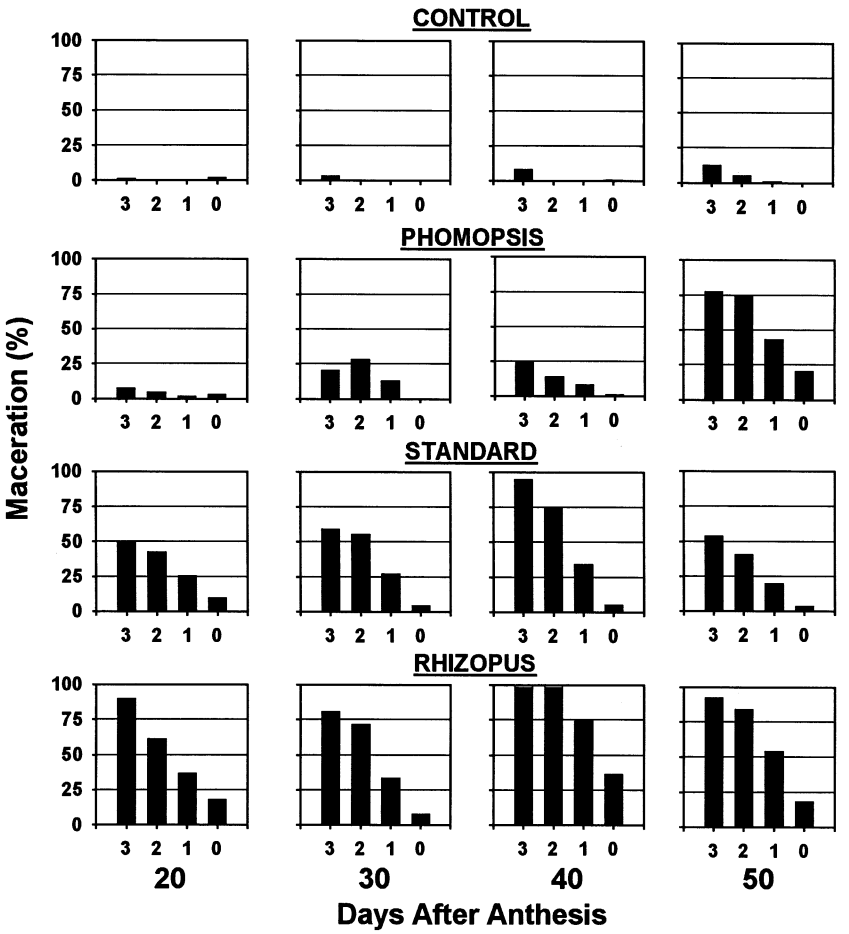

Fig. 2. Percentage maceration of muskmelon fruit tissue types at 20, 30, 40 and 50 day post-anthesis, fruit being subjected to partially purified polygalacturonase (PG) enzymes of *Phomopsis cucurbitae* and *Rhizopus stolonifer* as compared to a standard PG and the control. Numbers represent fruit tissue type: 0, approximately 2 mm thickness of exocarp; 1–3, fruit mesocarp divided into thirds with 3 being the inner mesocarp.

4. Discussion

The plant cuticle is the first line of defense to infection by fungal pathogens. The cuticle is composed of an insoluble biopolyester cutin that is embedded in a complex mixture of hydrophobic materials collectively called wax. It has been suggested that disease resistance in muskmelon fruit is enhanced by the presence of a surface cuticle and the development of suberized cork cells in the net (Davis et al., 1965; Yamamoto et al., 1995). However, in the netted muskmelon, the cuticle provides little protection between 12 and 25 days post-anthesis due to epidermal disruption in the initial stages of net development (Bruton, 1995). Once the cuticular barrier is breached by the fungus, further colonization may progress unimpeded as is generally the case with wound pathogens, or colonization may be arrested in the case of latent infections. However, *R. stolonifer* has not been reported to cause a rot on immature fruit during net development, possibly due to the lack of nutrients and a favorable environment.

In the present study, *R. stolonifer* produced approximately 80% more decay in inoculated mature muskmelon fruit compared to *P. cucurbitae*, yet, *R. stolonifer* produced two PG isozymes as compared to nine for *P. cucurbitae*. The number

Table 1
Regression coefficients, intercepts, slopes, and R^2 values for pectolytic enzyme maceration of different muskmelon fruit tissue types compared to controls and the commercial standard over time

Enzyme	Regression coefficients					
	Day	Intercept	Stderr	Slope	Stderr	R^2
Control	20	−0.4606	0.78	0.4198	0.42	0.02608
	30	0.9136	0.95	−1.5027	0.51	0.18756
	40	6.0824	1.35	−2.3595	0.73	0.21740
	50	11.8559	0.96	−5.0599	0.51	0.71836
Phomopsis	20	2.5310	0.99	−2.4271	0.53	0.53791
	30	25.9565	2.31	−6.5152	1.28	0.55185
	40	23.0769	0.86	−6.9702	0.46	0.92732
	50	57.2348	2.28	−11.7749	1.22	0.83806
Standard	20	52.5625	1.21	−13.7164	0.64	0.92264
	30	52.6435	1.14	−14.8008	0.57	0.88605
	40	67.0868	1.31	−19.3653	0.70	0.95241
	50	46.2533	1.26	−13.3518	0.67	0.91205
Rhizopus	20	58.4071	1.21	−12.7871	0.65	0.96520
	30	55.0036	1.77	−12.4333	0.94	0.92538
	40	82.6475	0.77	−17.7080	0.41	0.99040
	50	58.7787	1.80	−11.3381	0.96	0.88492

of PG isozymes produced does not appear to be directly related to decay aggressiveness when comparing virulence between the two fungi. Several other investigators have reported PG production by *R. stolonifer* (Cappellini, 1966; Pratt, 1971; Lee and West, 1981; Swinburne, 1983). The two PG isozymes produced by *R. stolonifer* in the present study had p*I* values of 6.2 and 7.9, respectively. Although postharvest decay caused by *P. cucurbitae* has been responsible for considerable losses (Bruton, 1995), there is little information available on the pathogen. The fungus has been reported to produce PG and β-galactosidase on autoclaved fruit exocarp tissue (Bruton and Biles, 1995). In addition, *P. cucurbitae* can cause a vine decline of muskmelon (Bruton, 1996a), producing PG, β-galactosidase, and cellulase on autoclaved muskmelon root tissue (Bruton et al., 1996).

There is little information available on the mechanisms involved in the regulation of latent infections. Verhoeff (1974) suggested three possible mechanisms involved in latent infections: (1) toxic compounds that inhibit the pathogen are present in unripe but not in ripe fruit; (2) unripe fruit do not provide a suitable substrate to fulfill the nutritional and energy requirements of the pathogen; and (3) the enzyme 'potential' of the fungus is inadequate to colonize unripe fruit. The nutritional requirements of the pathogen and enzyme potential of the fungus can be eliminated in the present study since the respective fungi had been deleted from the system.

Fruit softening and senescence following harvest is thought to be directly or indirectly related to the reactivation of latent infections (Prusky, 1996). It is generally accepted that fruit softening involves cell wall changes due to enzymatic hydrolysis by various cell wall hydrolases, although other factors such as non-enzymatic pectin solubilization or qualitative changes in cell wall synthesis, may also be involved (Seymour and Gross, 1996). It is apparent that in muskmelon fruit, PG is not involved in textural changes, since PG activity has not been demonstrated in muskmelon fruit (Lester and Dunlap, 1985; McCollum et al., 1989; Bruton, 1995). Gross and Sams (1984) reported that muskmelon fruit cell walls lost substantial amounts of galactosyl and smaller

Table 2
Cell wall carbohydrate composition of muskmelon fruit exocarp in relation to fruit age

Fruit age (days)	Rha	Ara	Xyl	Man	Glc	Gal	Galacturonic acid	Cellulose	Total
10	1.1a	2.6d	4.1a	1.3a	1.5a	12.5a	22.9b	26.4ab	72.5ab
20	1.2a	4.0a	4.7a	1.3a	1.6a	8.8b	27.1a	28.0a	76.96a
30	1.1a	3.7ab	4.6a	1.1ab	1.5a	4.7c	27.0a	23.4b	67.1c
40	1.1a	3.3bc	4.3a	1.2ab	1.5a	2.8cd	27.1a	27.8a	69.2c
50	1.1a	3.0cd	4.5a	1.1ab	1.5a	2.0d	26.6a	25.8ab	65.4c

Values are means of at least three determinations and are expressed as mg/100 mg of cell wall material.

amounts of arabinosyl residues during maturation and ripening. They further noted that galactose was the primary non-cellulosic neutral sugar component of melon fruit cell wall. Fils-Lycaon and Buret (1991) classified the various glycosidases detected in melon mesocarp into three groups: Group A, composed of α-galactosidase, α-mannosidase and α-arabinofuranosidase, whose activities decreased during ripening; Group B, composed of β-galactosidase, β-glucosidase and α-arabinopyranosidase, whose specific activities increased toward the end of ripening and overripening; and Group C, composed of β-galactosidase, whose specific activity remained the same from pre-ripe through the overripe stage. Ranwalla et al. (1992), demonstrated that muskmelon β-galactosidases are involved in the modification of both pectic and hemicellulosic polymers and are at least partially responsible for fruit softening.

In the present study, the PG enzymes were highly purified to the greatest extent possible. The following evidence suggests that the tissue maceration was due to fungal PG: no pectin lyase or pectate lyase activity was detected in samples (data not shown); both *R. stolonifer* and the standard PG exhibited similar maceration patterns and the commercial PG was from a *Rhizopus* species; and any contaminating enzymes would have to co-chromatograph with the PGs. PG isozymes from *R. stolonifer* and the commercial standard had much the same tissue maceration pattern on 20–50-day post-anthesis fruit. Conversely, PG isozymes from *P. cucurbitae* macerated little tissue until melons were 50 days post-anthesis fruit. Zhang et al. (1997) reported that *P. cucurbitae* produced much higher PG activity and a greater number of PG isozymes on mature fruit tissue as compared to immature fruit tissue. These data may suggest that inhibition of *P. cucurbitae* PG, not necessarily the fungus proper, may be an important determinant in latent infections. Differential inhibition of pectic enzymes has been observed among host proteinaceous inhibitors of soft-rotting fungal enzymes (Brown and Adikaram, 1982; Abu-Goukh and Labavitch, 1983; Brown, 1984). The concentration of these inhibitors decreases as fruit mature at

Table 3
Non-cellulosic neutral sugars and galacturonic acid present in fungal decayed tissue of muskmelon fruit

Treatment	Neutral sugars (μg/mg CW)[a]							Galacturonic acid (μg/mg CW)
	Rha	Ara	Xyl	Man	Gal	Glc	Total	
Control	6.0	17.8	46.1	6.5	36.7	15.9	129.0	298.7
Phomopsis	ND[b]	1.9	37.9	18.0	34.4	100.2	192.4	43.6
Rhizopus	ND	1.8	50.1	10.7	22.5	26.0	111.1	36.0

The fruit were inoculated at 40 days post-anthesis and incubated for 7 days.
[a] Values are means of at least three determinations.
[b] ND, none detected.

which time the fruit become more susceptible to decay (Abu-Goukh and Labavitch, 1983). Our data suggests that inhibitor(s) may be present in immature muskmelon fruit which may be involved in decay resistance and differentially inhibitory to the PG isozymes produced by *R. stolonifer* and *P. cucurbitae*.

In addition, the fact that substantial amounts of non-cellulosic neutral sugars, primarily rhamnosyl and arabinosyl with lesser amounts of galactosyl residues, were removed from the melon cell walls during fungal decay suggests that the fungal PGs act on highly branched pectins in the cell wall (Seymour and Gross, 1996). Thus, differences in ability of these fungal enzyme extracts to macerate melon cell wall from different maturities, and therefore different cell wall carbohydrate compositions, may reflect differences in the fungal hydrolytic enzyme complement present, or perhaps difference in the substrate specificities of the PGs produced by the two fungi.

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